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Differentiating greenbug resistance genes in barley

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Abstract The greenbug [*Schizaphis graminum* (Rondani)] is an extremely damaging pest of barley (*Hordeum vulgare* L), particularly in the southern Great Plains of the USA. Two greenbug resistance genes, *Rsg1a* (in 'Post 90') and *Rsg2b* (in PI 426756), available for developing resistant barley cultivars, have similar phenotypes when challenged by various greenbug biotypes. This study was conducted to separate these two resistance genes via differential plant reactions to a recently collected field isolate of greenbug. Four barley entries and one wheat germplasm were challenged with two greenbug isolates and damage ratings were recorded for each combination. One greenbug isolate used in this study (TX1) was able to differentiate *Rsg1a* from *Rsg2b* through dramatically different plant responses (*Rsg2b* conferred resistance, *Rsg1a* did not). The results indicate the potential vulnerability of greenbug resistance genes in barley. Based on these and other reported results, we propose that gene symbol designations for greenbug resistance in barley be changed from *Rsg1a* to *Rsg1* and *Rsg2b* to *Rsg2*.

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The greenbug is an economically important pest of barley in the U.S., particularly in the southern Great Plains where damaging populations are present every year (Starks and Webster 1985). Currently, there are two greenbug resistance genes in barley (*Rsg1a* and *Rsg2b*) available for developing greenbug resistant cultivars (Merkle et al. 1987).

Rsg1a is a single dominant gene first detected in PI 87181 and previously referred to as *Grb* (Gardenhire and Chada 1961). *Rsg1a* is located on linkage group 1 and on the centromere-bearing segment of chromosome 1 in the T1-6a translocation of 'Will' barley (Gardenhire et al. 1973). Will barley was used as a parent to develop 'Post,' which was later found to be heterogeneous for greenbug resistance, so a composite of individual plant selections was made, and a homogeneous greenbug-resistant winter barley was released as 'Post 90' (Mornhinweg et al. 2004). *Rsg2b* is also a single dominant gene (Merkle et al. 1987) discovered in PI 426756 (Webster and Starks 1984). Genetic studies showed that this new source of resistance was nonallelic to *Grb*, thus the gene symbol *Rsg2b* was assigned to this gene, and *Grb* was modified to *Rsg1a* (Merkle et al. 1987).

These two resistance genes provide protection against a variety of greenbug biotypes (B, C, E, F, G, I and K) (Porter and Mornhinweg 2004a, b) and have been the only sources of resistance reported to date. The responses of Post 90 (*Rsg1a*) and PI 426756 (*Rsg2b*) to challenges by all greenbug biotypes tested to date were rated as moderately resistant or resistant (Porter and Mornhinweg 2004a, b). While differences in the levels of resistance conferred have been detected between the two genes, no clear differentiation of plant phenotype has been elicited from a given greenbug biotype (e.g., virulent to *Rsg1a*, avirulent to *Rsg2b*).

Tyler et al. (1987) stated that criteria for resistance gene designations include evidence of inheritance as a single gene and evidence of uniqueness (i.e., ability to be distinguished from other resistance genes via different phenotype or different species origin of the gene). While evidence of single gene control has been documented (Merkle et al. 1987), clear separation of phenotypes for *Rsg1a* and *Rsg2b* in response to greenbug feeding has not been reported. The objectives of this study were to differentiate responses of barley plants carrying resistance genes *Rsg1a* and *Rsg2b* by challenging with a greenbug isolate recently collected from the field, and to propose renaming these genes in accordance with prevailing gene designation conventions.

Materials and methods

Four barley entries (Post 90, PI 426756, 'Wintermalt', and 'Colter') and one wheat entry (GRS 1201) were used in this study. Post 90 carries the *Rsg1a* resistance gene. PI 426756 carries the *Rsg2b* gene. Wintermalt and Colter were included in this study due to their divergent reactions to biotype G, or as susceptible checks (Porter and Mornhinweg 2004a, b). The wheat germplasm GRS 1201 was included as a resistant check to verify identity of the greenbug isolates used (Porter et al. 1997). Each barley and wheat entry was tested against one reported biotype (E) and one greenbug collection made from the field (TX1). TX1 was collected in Potter County, TX in 2003. This greenbug isolate was selected because it was found to have a distinctively different plant damaging phenotype in preliminary tests (data not shown, 2005).

Five seeds of each entry were planted (2 cm deep) in 3.8 cm diameter cells spaced 3.8 cm apart within rows and 3.8 cm between rows (replicated six times) in a flat ($30 \times 50 \times 4.5$ cm) containing Redi-earth Peat-Lite mix (Scotts-Sierra Horticultural Products Company, Marysville, OH). There was a total of 30 cells planted (5 entries with 6 replications) in a randomized complete block design, with a row of cells planted with 'Shuyler' barley planted between test entry rows to fill out the remaining cells in the flat. Two flats were planted and each tested with one of the two greenbug isolates. Standard greenbug culture and resistance evaluation protocols were used (Starks and Burton 1977). The tests, conducted in a greenhouse, were planted on 6 September 2005, infested on 9 September 2005, and damage ratings were recorded on 28 September 2005. A composite damage rating (1 = no damage, to 9 = dead plant) was recorded on each group of five seedlings per entry when the susceptible check rated a 9.0 (i.e., dead plant). Characterization of damage scores was as follows: 1–3 (resistant), 4–6 (moderately resistant to moderately susceptible), 7–9 (susceptible). Data from the test were subjected to Kruskal-Wallis one-way nonparametric analysis of variance, and the comparison of mean ranks was conducted at the 0.05 rejection level.

Results and discussion

Mean damage ratings and mean ranks of all entries tested against the two greenbug isolates are listed in Table 1. Significant differences ($P < 0.001$) were observed among entries for damage ratings. Post 90 and PI 426756 were both resistant to greenbug biotype E with a damage rating of 3.0 and 2.3, respectively. Wintermalt and Colter were both highly susceptible with a damage rating of 9.0. The resistant control, GRS 1201, was highly resistant to biotype E (Table 1). While PI 426756 was slightly more resistant than Post 90

Table 1 Damage ratings of four barley entries and one wheat germplasm tested against two greenbug isolates>

Entry	Gene designation	Greenbug isolate			
		E		TX1	
		Damage rating[a]	Mean Rank[b]	Damage rating	Mean rank
Post 90	*Rsg1*	3.0	14.5 ABC	9.0	21.5 B
PI 426756	*Rsg2*	2.3	10.2 AB	1.5	7.0 A
Wintermalt		9.0	24.0 BC	9.0	21.5 B
Colter		9.0	25.0 C	9.0	21.5 B
GRS 1201	*Gb6*	1.3	3.8 A	1.3	6.0 A
Critical Z value (0.05%)			2.8		2.8
Critical value for comparison (0.05)			14.3		14.3

[a] 1 = no damage, 9 = dead plant.

[b] Kruskal-Wallis mean ranks within column followed by the same letter are not significantly different

to biotype E (2.3 versus 3.0, respectively), they could not be separated by their Kruskal-Wallis mean rank. This is consistent with other reports of the reactions of these two resistance genes to various greenbug biotypes (Porter and Mornhinweg 2004a).

In contrast to biotype E, the greenbug isolate TX1 was able to severely damage Post 90 (*Rsg1a*). TX1 killed Post 90, Wintermalt, and Colter, while PI 426756 (*Rsg2b*) was scored highly resistant (damage rating of 1.5). The resistant control, GRS 1201, maintained its resistance against this otherwise highly virulent greenbug isolate with a damage rating of 1.3 (Table 1). Thus, the greenbug isolate TX1 was able to clearly differentiate between *Rsg1a* and *Rsg2b*, highlighting their unique genetic status.

Although the greenbug isolate TX1 was collected from Johnsongrass (*Sorghum halpense* L. Pers), it has the potential to infest barley production throughout the southern Great Plains. One way to prepare for this possibility and provide broad-spectrum protection is to simply combine both resistance genes into a common barley background.

Although Merkle et al. (1987) reported that these two single genes were nonallelic and independent, a more convincing test for separating resistance genes is through differential plant reactions to greenbug isolates. As indicated in Table 1, Post 90 (*Rsg1a*) reacts differently than PI 426756 (*Rsg2b*) when challenged by greenbug isolate TX1. These results complement previous reports of independent genetic control and confirm the uniqueness of the two resistance genes. As such, a modification of the gene symbol designation of these two genes is in order. Under current rules for nomenclature and gene symbolization in barley, *Rsg1a* and *Rsg2b* denote alleles of *Rsg1* and *Rsg2*, not the genes themselves (Franckowiak and Lundqvist 2004). Given that *Rsg1a* and *Rsg2b* are unique and nonallelic, we propose that their gene symbol designations be changed to *Rsg1* and *Rsg2*, respectively.

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